Soil Microorganismus and Activators.*

By

Arao Itano.

[Aug. 13th, 1926]

Introduction.

In the light of marked progress made recently in the studies of nutrition, especially in regard to so-called activators including vitamins, the members of the Protista[1] received a great deal of attention as well as those of the animal and plant kingdom. In fact the new interpretations have been made in regard to their physiological activities and phenomena of which nature hitherto unknown. It is the purpose of this paper to deal with the general survey of this field concerning the soil microorganisms in particular together with some results obtained by the author, and also discuss briefly the future scope.

General Survey.

Previous to the discovery of vitamins[2], the influence of external factors viz. activators on the action of enzymes were investigated extensively. Since the enzymes have such intimate connection with the metabolic process in general and especially with that of microorganisms, the investigations in the field of microbiology have been influenced to a great extent. For example, in 1897 BERSTRAND[3] reported the action of manganese on the laccase and named the salt as co-ferment, and later in 1912[4] he investigated the influence of the same salt upon Aspergillus niger and noted its stimulating effect. However as to the exact nature, accessory or required, of the salt in his latter experiment is subject to question since the amount used was much larger than that of sugar and ammonium salts which are generally considered as the required food for the organism employed. Of course it is generally conceived that it is very difficult to draw a distinct line of differentiation between the accessory and required food for microorganisms since the minimum food requirement is very small and varied beside the lack of the fixed criteria.

1) MINCHIN, E. A. Introduction to the Study of the Protozoa.
2) FUNK, SUZUKI and others.
3) BERSTRAND, G. Compt rendus, CXXIV, 1032, 1355, 1897.
4) ibid , CLIV, 616, 1912.

* Published in the Journal of the Agricultural Chemical Soc. of Japan, 1, 13, 1029, October 1925.

Again what have been found as to the influence of hydrogen ion concentration on the enzymic activities[1] were introduced into the field of microbial studies which will be discussed further in detail in connection with the author's investigation.

Since the discovery of vitamins, a new field of study was created for the microbiologists as well as for the others and we find numerous investigations. Consequently the new interpretations were brought out in regard to some physiological phenomena which remained as mystery. For example, as early as 1872 PASTEUR[2] noted a phenomenon which he termed "auto-association", in connection with his study of the alcoholic fermentation. Later in 1901, WILDIERS[3] interpreted the phenomenon presuming the presence of a hypothetical nutrient to which he gave the name, "bios". As the knowledge of vitamin advanced, the similarity between the "bios" and vitamin B was pointed out, but later Funk and his co-worker[4] designated it as vitamin D as the result of their investigations. Very recently Suzuki and his co-worker[5] proposed to call the similar substance as "Biogeninsaure" because they consider it more appropriate as well as it avoids confusion.

Besides the yeast, numerous other microorganisms especially the pathogenic bacteria[6] such as tuberculosis, meningococcus, influenza typhoid and others have been studied in connection with their vitamin requirement. However the direct investigation on the soil microorganisms is very scarce up to the present time. In fact the presence of growth promoting substances in Azotobacter[7] and a few other microorganisms have been investigated to some extent. But in regard to the influence of these substances upon the soil microorganisms, only a few investigations are known to the author. BOTTOMLEY[8] carried out a bacterial test for plant food accessories (Auximones) and found that Azotobacter chroococcum fix the nitrogen almost three times as much as they do without it. His results were substantiated by MOCKERIDGE[9] later.

Some Results of the Author's Investigations.

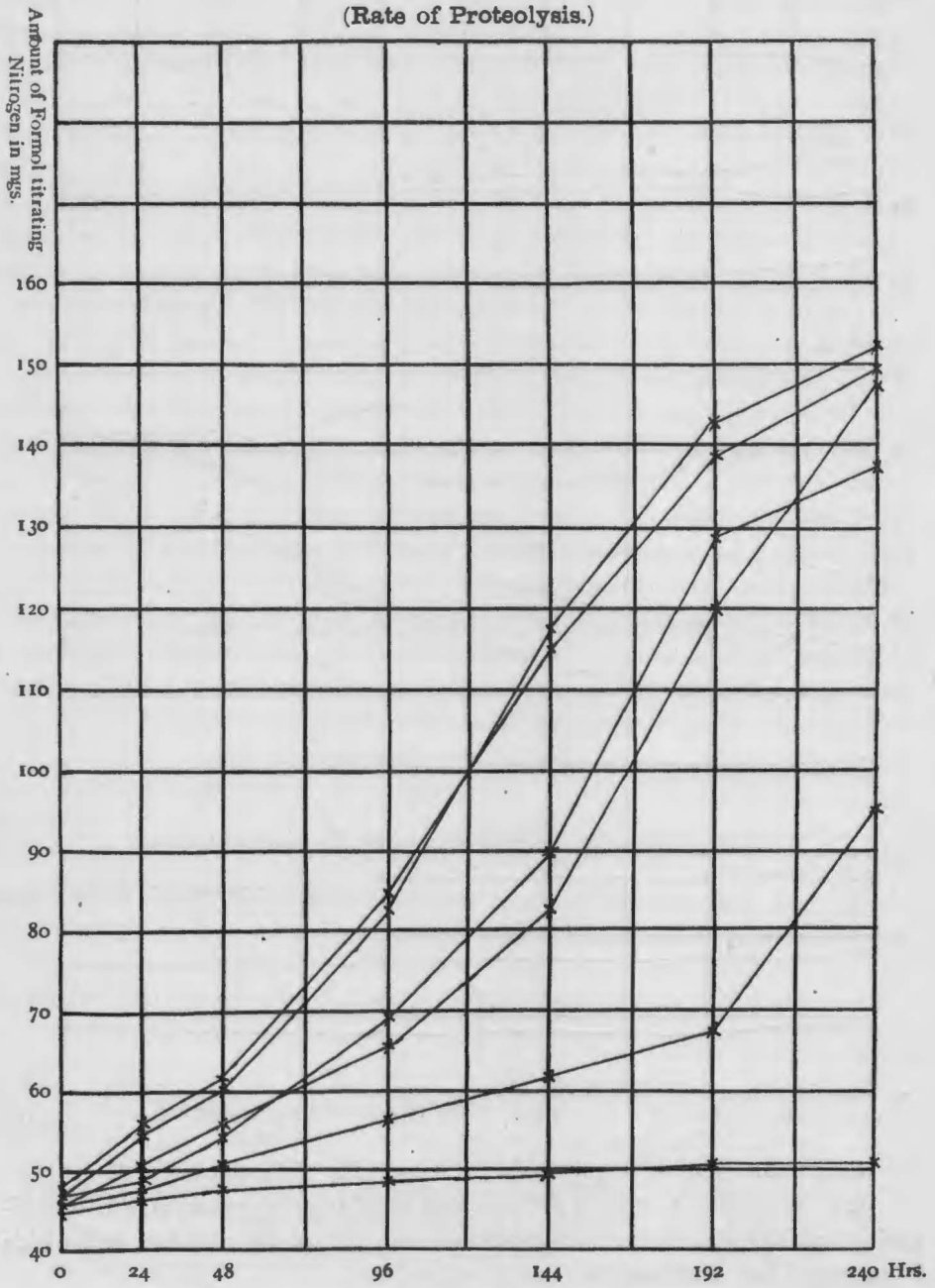
I. The Relation of Hydrogen Ion Concentration of Media to the Proteolytic Activity of Bacillus Subtilis: (1916)[10]

1) SORENSEN S. P. L., Biochemie Zeitschr. 7, 45, 1907; Compt. rend. du. Lab. de. Carlsberg 7, 1 etc.
2) PASTEUR L., Ann. Chem. Phys. 25, 45, 1872.
3) WILDIERS, La Cellule, 18, 313, 1901.
4) FUNK C. and DUBIN H. E., J. Biol. Chem. 48, 437, 1921.
5) SUZUKI B. and T. TAIRA, J. Chem. Soc. Japan, XLV, 299, 1925.
6) FUNK C., Die Vitamins, 1922, S. 37.
7) ISSATSCHENKO B., Centbl. f. Bakt. II, 57 (1922) 271.
8) BOTTOMLEY W. B., Proc. Royal Soc., London, B. 88, 1914, 237.
9) MOCKERIDGE F., ibid , B. 89, 1917, 508.
10) ITANO A., Mass. Agr. Exp't Staiton, Bull. 167, 1916.

In this investigation, the author undertook to ascertain the influence of hydrogen ion concentration on the vitality, growth, rate of proteolysis of B. subtilis and also the nature of the enzyme concerned in the process. Some of the results are gived here.

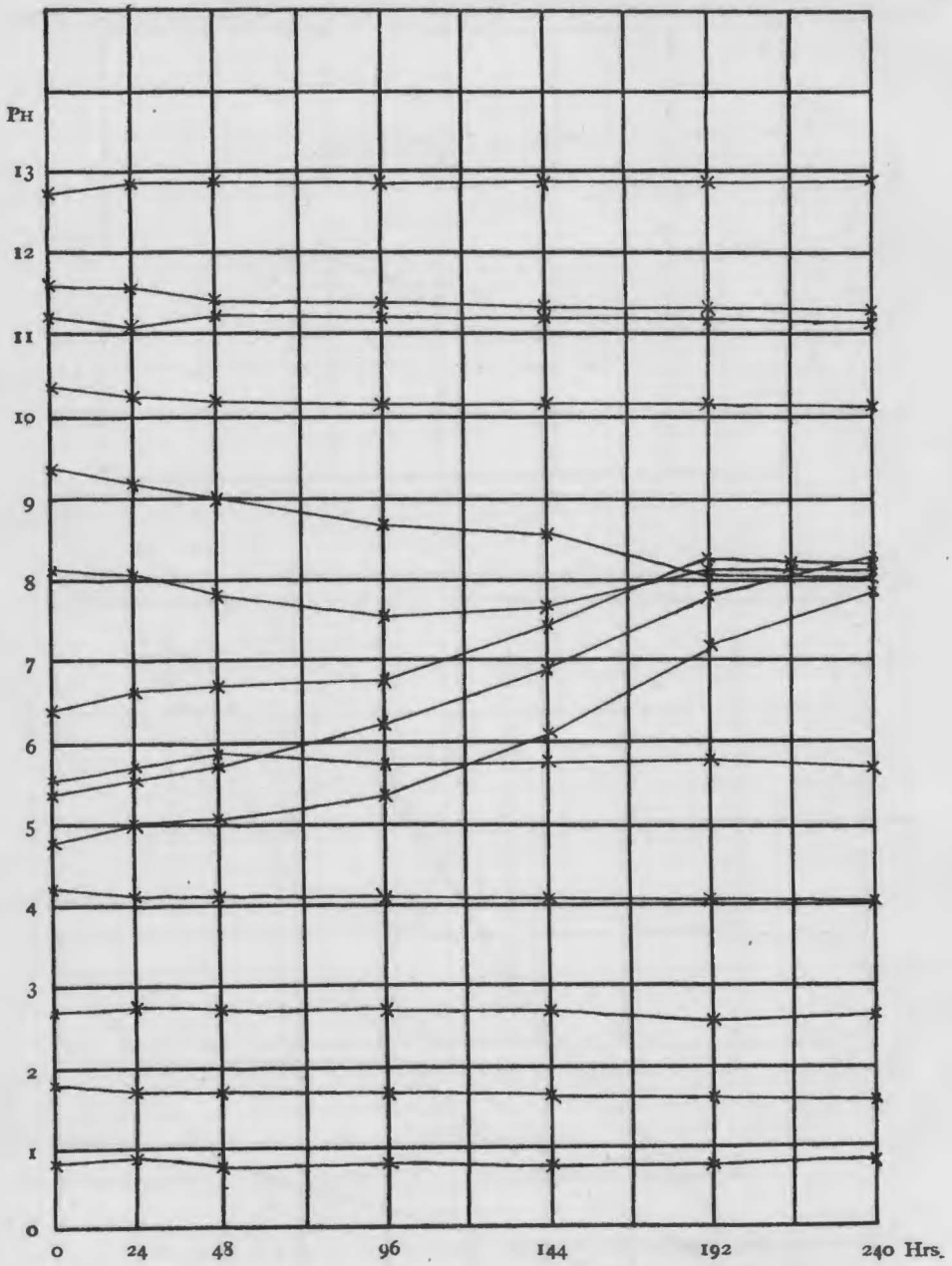
Graph A.

(Rate of Proteolysis.)



Graph B.

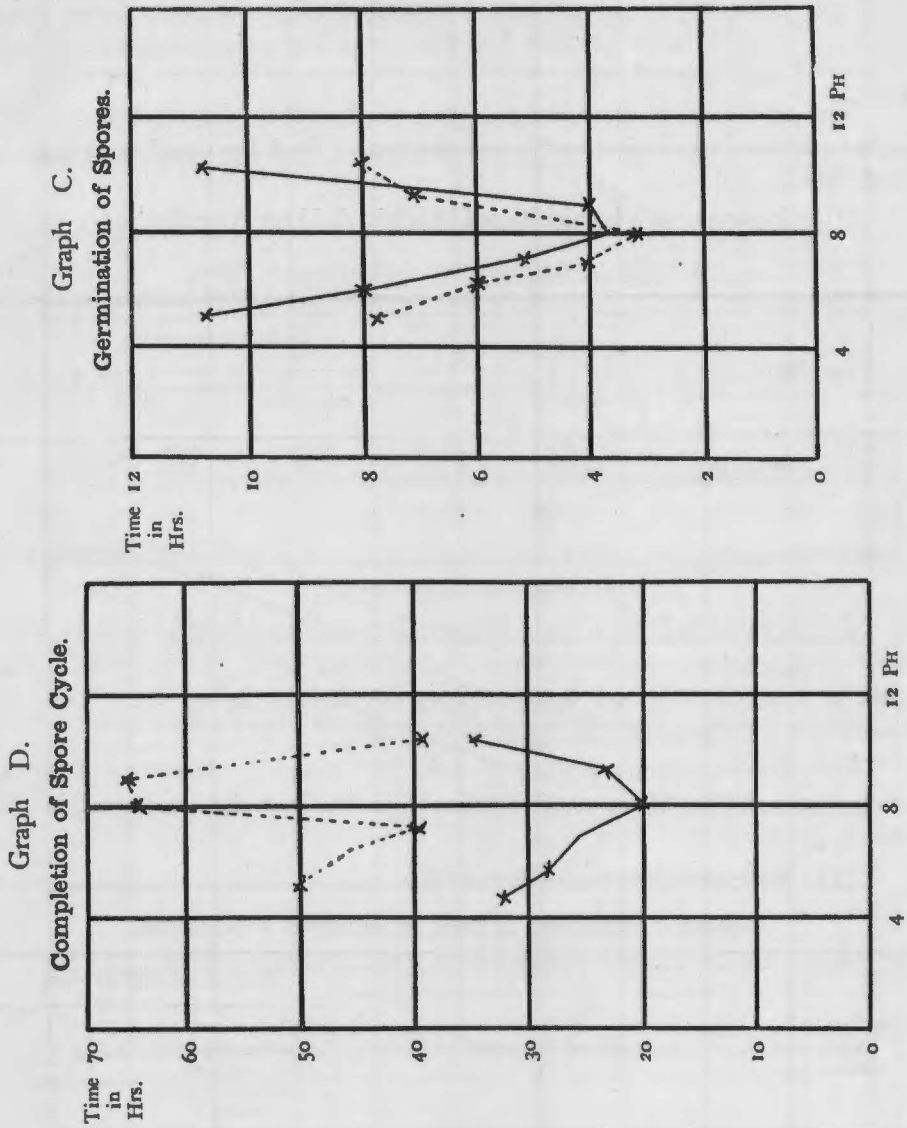
(Automatic adjustment of H Ion Concentration in media.)



Graph A. Rate of Proteolysis. Graph B. Alteration of PH.

It is interesting to note a phenomenon which was observed in this investigation and indicated as an " automatic adjustment " of the reaction, and which is presented here in Graph B.

II. Influence of Temperature and Hydrogen Ion Concentration upon the Spore Cycle of Bacillus Subtilis: (1918)[1]



Graph C. Germination of Spores. Graph. D. Completion of the Spore Cycle.

The results obtained in this investigation are shown in the graphs:

1) At 25°C and at 37°C germination occurs within PH 5—10,.
2) The spore cycle is completed only within PH 5—10,.
3) The spores can germinate in PH 10, although after germination the

1) ITANO A. and J. Neill, J. Gen. Physiology, I, 2, 421, 1919.

vegetable cells multiply only to a very slight extent and soon pass into spores.

4) The range of hydrogen ion concentration for spore germination seems to be greater than the range for active vegetable growth and multiplication.

The results given here are not only of theoretical importance but they have a practical application to the preservation of food by canning as well as to the similar industries.

III. Influence of Vitamin B? and Nucleic Acid on Azotobacter[1]: (1923).

Growth of Azotobacter and nitrogen fixed.

Media	Number of orgaisms per cubic centimeter.		N per 100 cc.		Gained
	Initial	After ten days	Initial N	After ten days	
		millions	mgm.	mgm.	
Ashby + vitamine B(?) ...	19.000	1.550	0.2	15.0	14.8
Ashby + phytonucleic acid	19.000	1.250	0.2	13.0	12.8
Plain Ashby	19.000	450	0.2—	5.7	5 5

As the table indicates, both vitamin (B?) and nucleic acid increased the number of organisms and the amount of nitrogen fixed. It is apparent that there is a slight difference in the degree of stimulation by these two, the former seems to be more effective than the latter.

This investigation particularly vitamin (B?) should be investigated further as to the possible presence of vitamin D in the material employed in this investigation.

IV. Biological Investigation of Peat[2].

Change of nitrogen in peat by different treatments.

Number of flasks	Control and Treatment	Per cent of total nitrogen	
		Alkaline permanganate soluble nitrogen (A. O. A. C. method)	Amino acids (Van Slyke)
Control	1 gram of peat + 5 c.c. H_2O	5.90	0.50
1.	Control + m_8 NaOH to reaction PH 7.0	36.75	6.80
2.	Control + m_8 Na_2HPO_4	33.50	5.70
3.	Control + $CaCO_3$	32.00	4.60

1) ITANO A., J. Bact. 8, 5, 1923.
2) ITANO A., J. Bact. X, 87, 1923.

Number of flasks	Control and Treatment	Per cent of total nitrogen	
		Alkaline permanganate soluble nitrogen (A. O. A. C. method)	Amino acids (Van Slyke)
Control	1 gram of peat + 5 c.c. H_2O	5.90	0.50
4.	Control + (1 : 1,000) vitamin B	33.50	5.80
5.	Control + (1 : 100) molasses	43.00	11.00
6.	Control + m_5NaOH + (1 : 1,000) vitamin B ...	65.70	20.00
7.	Control + m_5NaOH + (1 : 100) molasses	55.00	18.50
8.	Control + (1 : 100) vitamin B + (1 : 100) molasses	45.50	12.50
9.	Control + m_5NaOH (1 : 100) vitamin B + (1 : 100) molasses	71.50	25.50

As the table indicates, the amount of the alkaline permanganate soluble nitrogen and also amino acids in the peat was increased markedly by the addition of vitamin with other materials which seemingly were absent at the beginning.

Summary and Conclusions.

As the foregoing investigations indicate that the ecological factors influence the physiological activities of the soil microorganisms markedly. Especially the results obtained in the last investigation are noteworthy since the natural flora received the serious consideration.

I feet that in the field of soil microbiology more extensive as well as intensive investigations in regards to the ecological factors auch as the activators etc., should be carried out especially under the natural conditions as close as possible.